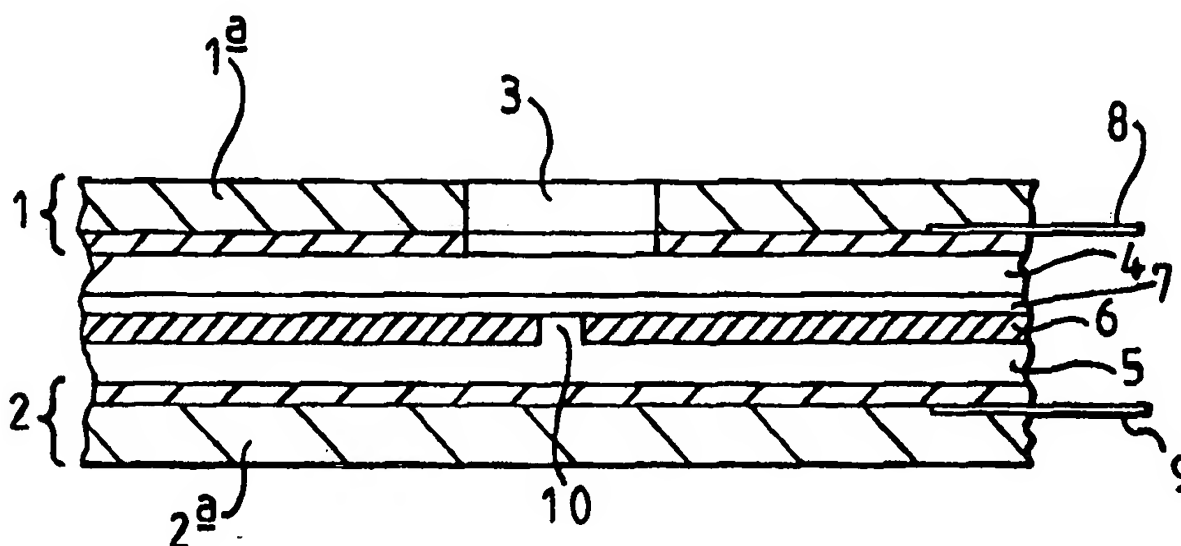




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(54) Title: BIOSENSOR COMPRISING A LIPID MEMBRANE CONTAINING GATED ION CHANNELS**(57) Abstract**

A biosensor has a lipid membrane (7) containing gated ion channels sensitive to the presence or otherwise of an analyte molecule in a sample applied, in use, to a first side of the lipid membrane (7). The lipid membrane (7) is disposed between a pair of electrodes (1, 2) in which a first layer of porous gel (4) is applied to the first side of the lipid membrane (7).

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BIOSENSOR COMPRISING A LIPID MEMBRANE CONTAINING GATED ION CHANNELS

This invention relates to a biosensor, and in particular to a biosensor of the type which operates by detecting or measuring the transport of ions across a lipid membrane.

Biosensors based on the use of a gated channel protein spanning a bilayer membrane are of
5 considerable interest. Each individual binding event can give rise to the passage of as many
as 10^9 individual ions through the channel during a practical measurement interval. Also, the
operation of distinct channels is essentially independent and the currents through them
combine linearly. These two factors inspire the hope of a general principle for sensing
biomolecules which displays both excellent sensitivity and high dynamic range.

10 In order to achieve high dynamic range it is necessary to choose channel proteins which open
in the presence of the target biomolecule. This is the case for a number of naturally-
occurring channel proteins, typically neurotransmitter receptors in nerve cells. In a more
generally applicable approach, robust ungated channels, particularly the gramicidins, are
bound chemically to antibody molecules in such a way as normally to obstruct the channel,
15 and to unblock it when an antigen binds.

The electric charge transported through these channel proteins consists physically of solvated
ions. In order to allow further processing, the ions must be exchanged for the flow of
electrons through a wire at electrodes located at both the front and rear of the bilayer. In one
known approach, the bilayer is located immediately adjacent to the rear noble-metal
20 electrode. It is not clear where the ions flow to. If they discharge at the electrode, the
associated chemical changes will inevitably lead to degradation.

In another known approach, there is electrolyte behind the bilayer contained in a gel. The
bilayer is fabricated by a standard technique across an aperture adjacent to the gel. The gel
provides some physical support for the bilayer, so that it is able to withstand quite vigorous

agitation of the test liquid. However the bilayer cannot be dehydrated and must be formed immediately prior to the measurement in the aqueous medium to be monitored. It is directly exposed to the medium and cannot withstand contact with a solid.

5 There has now been devised a novel form of biosensor based on measurement of ion transport across a lipid membrane which overcomes or substantially mitigates the disadvantages of known forms of such biosensor.

According to a first aspect of the invention, a biosensor comprises a lipid membrane containing gated ion channels sensitive to the presence or otherwise of an analyte molecule in a sample applied, in use, to a first side of said lipid membrane, the lipid membrane being
10 disposed between a pair of electrodes, wherein a first layer of a porous gel is applied to the first side of the lipid membrane.

The biosensor according to the invention is advantageous primarily in that the first layer of porous gel applied to the lipid membrane protects the membrane from dehydration and physical damage caused by mechanical contact, yet still permits molecules contained within
15 the sample access to the lipid membrane. Because the membrane is not destroyed by drying of the biosensor, the biosensor can be packaged and stored in the dry state, for rehydration immediately prior to use.

Preferably, a second layer of gel is also applied to the second side of the lipid membrane, to further protect the membrane and to provide the necessary separation from the adjacent
20 electrode and to accommodate a reservoir of ions required by that electrode.

The gel is preferably a biocompatible and porous gel, most preferably a hydrogel. Suitable gel materials include agarose, dextran, carrageenan, alginic acid, starch, cellulose, or derivatives of these such as eg carboxymethyl derivatives, or a water-swellaable organic polymer such as eg polyvinyl alcohol, polyacrylic acid, polyacrylamide or polyethylene
25 glycol.. A particularly preferred gel material is agarose. Other gel materials considered

particularly suitable include polyacrylamide gels.

The thickness of particularly the first layer of gel is preferably such as to permit diffusion of biomolecules of approximately 1kD to occur in reasonably short time periods, eg less than 5 minutes, more preferably less than 2 minutes. The first and second layers of gel preferably
5 have thicknesses of less than 5mm, eg 0.1 to 2mm, most preferably approximately about 1mm.

The lipid membrane is preferably a bilayer of amphiphilic molecules, most preferably one or more phospholipids, eg phosphatidylcholines and/or phosphatidylethanolamines. The lipids may have hydrocarbon tails with chain lengths of C_{12} - C_{22} , most preferably C_{12} - C_{18} . A
10 particularly preferred phospholipid is dioleoylphosphatidylcholine. Other membrane forming molecules which may be employed include amphiphilic polymers, eg hydrophobic polymer chains with hydrophilic side groups. One example of such a polymer is a polysiloxane with phosphatidylcholine side groups.

Suitable molecules defining the gated ion channels are incorporated into the lipid membrane,
15 eg membrane-bound proteins.

Preferably a perforated sheet of an inert and impermeable material is interposed between the lipid membrane and the second gel layer. A suitable such material is polytetrafluoroethylene. The sheet is preferably thin, eg less than about 100 μ m in thickness, more preferably about 10 μ m in thickness. The sheet is preferably formed with one or more perforations of diameter
20 10-200 μ m, more preferably about 50-100 μ m. The sheet of material permits the flow of current between the electrodes only in the region of the perforation(s) in the sheet.

The lipid membrane may be formed by dissolving the membrane-forming lipid and the molecules defining the gated ion channels in a solvent and applying the solution so formed to the second gel layer (or to the perforated sheet of inert material abutting the second gel
25 layer). Any suitable solvent may be used, provided that it is substantially immiscible with

water. Polar solvents, capable of initiating hydrogen bonds, are preferred since their use provides a strong driving force for complete coverage of the solution over the surface. A particularly preferred solvent is chloroform. The solution preferably has a concentration of 0.01 to 5% w/v, more preferably less than 1% w/v, eg about 0.2% w/v.

- 5 However, the method of forming the lipid membrane described above may not always be suitable. For example, some ligand-gated channel proteins may be denatured by chloroform. One alternative method for the formation of the lipid membrane which may be suitable in such cases involves forming an inverted micellar solution or emulsion containing the membrane forming lipid and molecules defining the gated ion channels, the micellar solution
10 or emulsion having a hydrocarbon continuous phase. The hydrocarbon is preferably an alkane, most preferably hexane.

One functional ligand-gated channel protein for which the alternative method described in the immediately preceding paragraph may be applicable is the nicotinic acetylcholine receptor (nAChR - see G Puu et al, *Biosens. Bioelectron.* 10 (1995), 463). This neuroreceptor, with
15 many slight variations, is found in most animals with nervous systems, and a very rich source of supply is available in the electric organ of the common marbled ray *Torpedo marmorata*. A crude extract formed by homogenizing the electric organ of the ray and centrifuging in a CsCl gradient to isolate the membrane-bound fraction has a continuous phase which is essentially aqueous. By reducing the proportion of water it is possible to invert the emulsion
20 and to prepare from it an inverse emulsion with a hydrocarbon continuous phase having the characteristics required for formation of the lipid membrane.

The electrodes are preferably noble metal electrodes of generally conventional form. Most preferably the electrodes are silver/silver chloride electrodes formed on sheets of a suitable substrate such as mica. Preferably the electrode on the first side of the lipid membrane is
25 formed with an aperture which serves as a sample introduction port. Where the device comprises a perforated sheet of material as described above, the aperture is preferably aligned with the perforation(s).

The invention further provides a method of qualitatively or quantitatively determining an analyte molecule in a sample, which method comprises applying the sample to the first layer of porous gel in a biosensor according to the first aspect of the invention.

5 The biosensor of the invention is most preferably assembled by coating each of two planar electrodes with a layer of porous gel, placing a solution or emulsion containing lipid molecules onto the second gel layer, and then placing one electrode on the top of the other such that the gel layers are disposed between them. Generally, the biosensor is assembled under the conditions such that the lipid membrane forms spontaneously. Monolayers of lipid will form at the interfaces between the solution or emulsion and the respective gel layers.

10 As the bulk solution or emulsion is expelled or evaporates from between the gel layers the two monolayers come together to form the lipid membrane bilayer.

A preferred embodiment of the invention will now be described in greater detail, by way of example only, with reference to the accompanying Figures, in which

Figure 1 is a schematic sectional side view of a biosensor cell according to the invention;

15 Figure 2 shows a bar histogram of cell resistances (logarithmic resistance scale) measured using a cell of the type shown in Figure 1, with and without a lipid bilayer; and

Figure 3 shows a scatter plot of measured cell resistance (log scale) measured using the cell of Figure 1 after long exposure to gramicidin, versus the time taken for the resistance to reach its ultimate value.

20 Referring first to Figure 1, a biosensor cell according to the invention is formed between a pair of planar Ag/AgCl electrodes 1,2 formed on mica substrates 1a,2a as described below. The upper mica substrate 1a has a 3mm diameter aperture 3 through which test samples may be introduced. The space between the electrodes 1,2 is filled by first and second layers 4,5 of agarose gel (prepared as described below) separated by a 10µm thick PTFE sheet 6 on the

upper surface of which is formed a lipid bilayer 7. The sheet 6 has at least one perforation 10. Silver wires 8,9 are connected to the electrodes 1,2.

Formation of Gel Sheets

A mixture of 1% by weight agarose, 10% by weight glycerol, 0.1M NaCl, 0.1M KCl and 5 0.01M CaCl_2 and the remainder ultrapure water were heated to boiling point. While still liquid the mixture was pipetted into a hydrophilic glass mould or surface (typically 6 ml) and allowed to set.

The gel sheets 4,5 were 1mm thick on initial formation. They were allowed to dehydrate completely under laminar flow of ambient air (nominal 23°C, 50% relative humidity). After 10 rehydration with electrolyte (0.1M NaCl, 0.1M KCl, 0.01M CaCl_2 in ultrapure water) the gel was removed from its substrate.

Formation of electrodes

The silver/silver chloride electrodes 1,2 were fabricated as follows. Freshly-cleaved pieces of mica 1a,2a were perforated as required (typically 3mm diameter holes in a piece of a few 15 centimetres in size). They were rinsed and sonicated separately with chloroform and methanol. A 12.5µm- diameter silver wire 8,9 was placed on the surface which was then coated with silver dag and allowed to dry, securing the wire 8,9. The surface was electrolytically chlorided in 1M HCl at 9V using a stainless steel counterelectrode, initially as the cathode for 10s and then as the anode for a further 10s. The rehydrated gel layer 4,5 20 described in the previous paragraph was placed in contact with the silver side.

Assembly of biosensor cell

10µm-thick PTFE film 6 was cut into cm-size pieces and perforated with a red-hot tungsten

tip. The resulting holes were typically 50-100 μ m in diameter.

A spreading solution was made up from L, α -dioleoylphosphatidylcholine at a concentration of 20g / ℓ in chloroform.

5 A structure shown in Figure 1 was assembled as follows. The perforated piece of PTFE 6 was placed on the lower electrode 2 with its gel coating 5. 10 μ l of spreading solution was spread over the PTFE 6 and a second, apertured electrode 1 with its gel coating 4 placed on top, ensuring that the aperture 3 was aligned with a perforation in the PTFE sheet 6.

Measurements

10 For measurement, the electrolytic bilayer cells produced by the above procedure were placed in a electrically-shielded box and a drop of test solution placed over the mica aperture. The resistance of the cells was measured with a Keithley Model 175 digital multimeter. To measure the variation of current as a function of time, the cell current was converted to a voltage using a Bio-Logic BLM-120 bilayer membrane amplifier with a transimpedance of 1.0G Ω . The output voltage was digitised by a Cambridge Electronic Design 1401 Plus
15 multichannel analyser and logged by a computer running the CDR program.

Figure 2 shows the results of six measurements of the resistance of the cell prepared as described above, contrasted with measurements made in the absence of a lipid membrane. As can be seen, the effect of the lipid membrane is to increase the measured resistance considerably.

20 Figure 3 shows measured resistance values for a cell prepared as described above, after long exposure to a 1g/ ℓ solution of gramicidin-D. The effect of the protein is to reduce the cell resistance.

Claims

1. A biosensor comprising a lipid membrane containing gated ion channels sensitive to the presence or otherwise of an analyte molecule in a sample applied, in use, to a first side of said lipid membrane, the lipid membrane being disposed between a pair of electrodes, wherein a first layer of a porous gel is applied to the first side of the lipid membrane.
5
2. A biosensor as claimed in claim 1 wherein a second layer of gel is applied to the second side of the lipid membrane.
3. A biosensor as claimed in any one of the preceding claims wherein the gel is a hydrogel.
10
4. A biosensor as claimed in any one of the preceding claims wherein the first layer of gel is such as to permit diffusion of biomolecules of approximately 1kD to occur in less than 5 minutes.
5. A biosensor as claimed in any one of the preceding claims wherein the lipids of the lipid membrane have hydrocarbon tails with chain lengths of C_{12-22} .
15
6. A method of assembling a biosensor comprising coating each of two planar electrodes with a layer of porous gel, placing lipid solution on to the second gel layer and then placing one electrode on top of the other such that the gel layers are disposed between them and are separated by a lipid membrane.
- 20 7. A method as claimed in claim 6 wherein prior to the formation of the lipid membrane the membrane-forming lipid and the molecules defining gated ion channels are dissolved in a solvent.

8. A method as claimed in claim 7 wherein the solvent is chloroform.
9. A method as claimed in claim 6 wherein, prior to the formation of the lipid membrane the membrane-forming lipid and molecules defining gated ion channels are incorporated into an inverted emulsion with an alkane-rich continuous phase.
- 5 10. A method as claimed in claim 9 wherein the alkane is hexane.
11. A method of qualitatively or quantitatively determining an analyte molecule in a sample, which method comprises applying the sample to the first layer of porous gel in a biosensor according to any one of claims 1 to 5.

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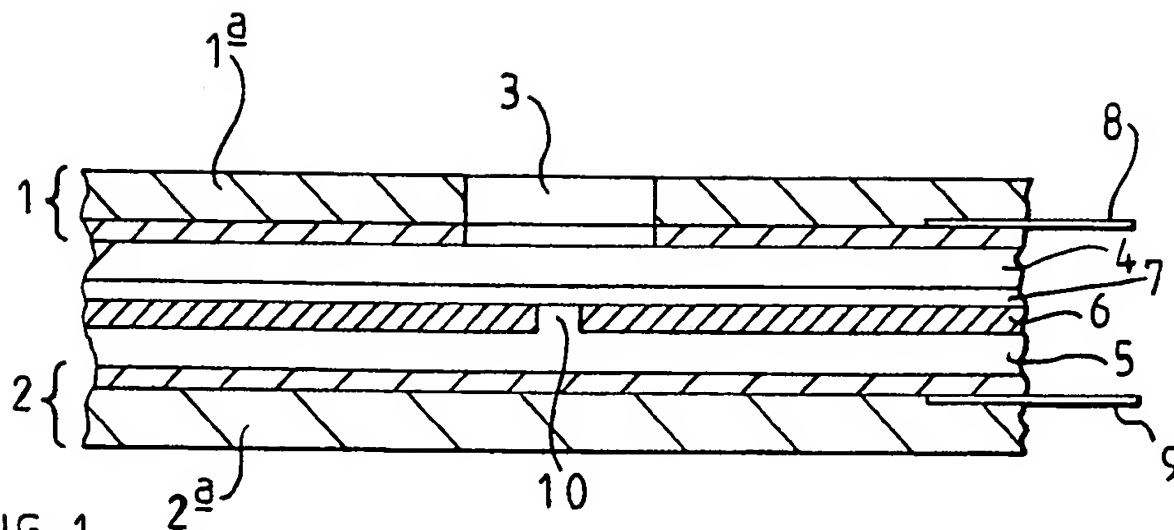


FIG 1

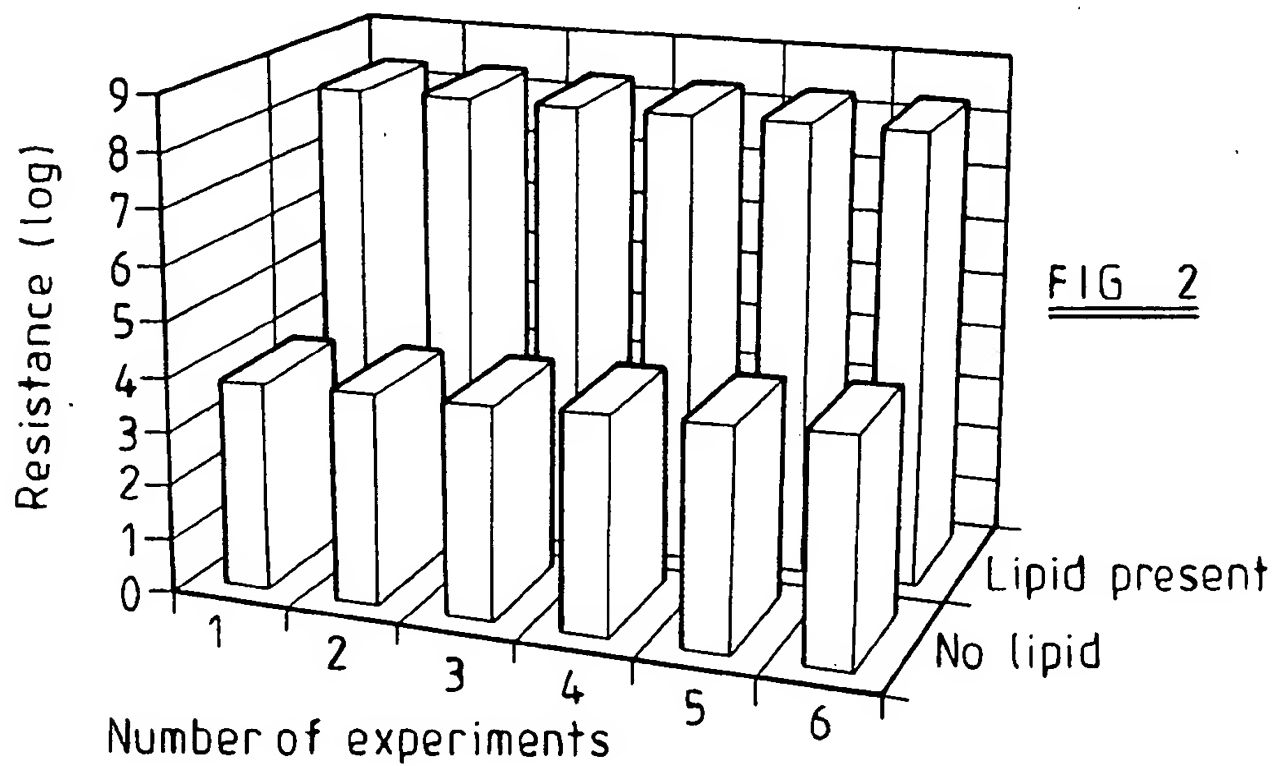


FIG 2

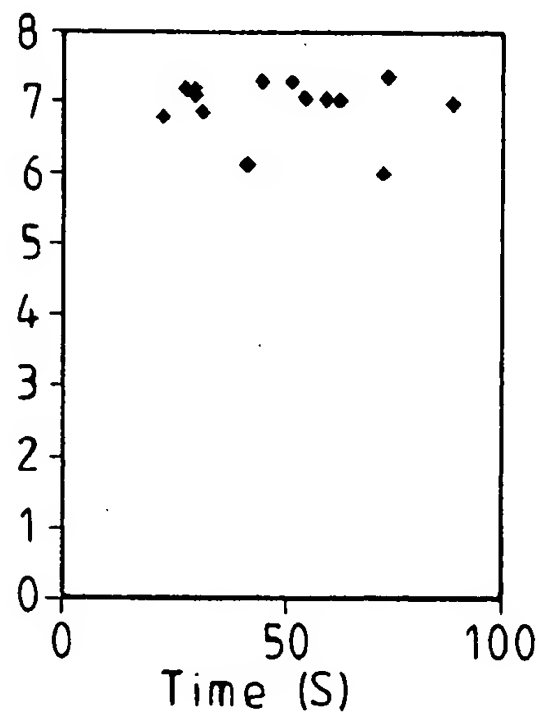


FIG 3